Functional Participation of the IL-2 Receptor γ Chain in IL-7 Receptor Complexes

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The γ chain of the interleukin-2 (IL-2) receptor is shared with the functional IL-4 receptor and is causatively related to X-linked severe combined immunodeficiency (XSCID), which is ascribed to a profound T cell defect. Studies with monoclonal antibodies specific for the IL-2 receptor γ chain showed that the γ chain participates in the functional high-affinity receptor complexes for IL-7 that are involved in the differentiation of T and B cells. Participation of the γ subunit in more than one receptor may enable the elucidation of the mechanisms of XSCID development and lymphocyte differentiation.

Cytokines mediating communication among cells in the immune system function through binding to specific receptors on target cells (*1*). Interleukin-2, a cytokine produced by T cells, interacts with the IL-2 receptor to induce T cell growth (*2*). The γ chain (*3*), in addition to the α and β chains, is essential for high-affinity IL-2 binding (*4*) and signal transduction (*5*). Mutations in the γ chain gene are closely associated with XSCID, a major feature of which is complete depletion of T cells (*6*), which demonstrates the indispensable function of the γ chain in T cell development.

We produced monoclonal antibodies (mAbs) to the γ chain of the mouse IL-2 receptor (*7*). When added to the culture of a mouse T cell line, CTLL-2, one of the mAbs [TUGm2; rat immunoglobin G2b (IgG2b)], which inhibits IL-2 binding to the high-affinity receptor, interfered with CTLL-2 cell growth that had been induced by IL-4, as well as growth induced by IL-2 (*7*). The other mAb (TUGm3; rat IgG2a), which does not interfere with IL-2 binding, detected the γ chain cross-linked with IL-4 (*7*). These data established that the γ chain is a shared component between the IL-2 and IL-4 receptors (*7, 8*). One may plausibly assume that the γ chain plays the exclusive role in the development of T cells as a component of the receptor complex for either IL-2 or IL-4. This does not, however, seem to be the case, because IL-2– or IL-4–deficient mice were found to be normal in terms of numbers of T cell subsets in the periphery (*9*). During our exploration of cytokines that require the γ chain as a component of their receptors, we found that the γ chain is included in the receptor complex for IL-7 that may be involved in T cell development (*10*).

We examined the effect of TUGm2 on the ligand-induced growth of mouse spleen cells (Fig. 1A). Interleukin-7 induced [^{3}H]thymidine incorporation by spleen cells that were incubated with concanavalin A (Con A) in a concentration-dependent fashion. When TUGm2 was added, the [^{3}H]thymidine incorporation in response to IL-7 was reduced. The inhibition effect was further examined with an IL-7–dependent pre–B cell line, IxN/2b (*11*). The IxN/2b cells also showed IL-7–dependent [^{3}H]thymidine incorporation that was inhibited by TUGm2 (Fig. 1B). The inhibition was most prominent at low concentrations of IL-7. Addition of a mAb, A7R34 (rat IgG2a), specific for the conventional IL-7 receptor (*12*) affected [^{3}H]thymidine incorporation of IxN/2b cells at IL-7 concentrations lower than 5×10^{-9} M, which was indistinguishable from incorporation without IL-7. Simultaneous addition of TUGm2 and A7R34 completely blocked the incorporation at any IL-7 concentrations tested. A control mAb, REY-7 (rat IgG2b), specific for the human T cell leukemia virus–type 1 (HTLV-I) envelope protein gp46 (*13*), did not have any detectable effect. Thus, IL-7–dependent growth required the function of the γ chain.

To examine the possibility that the γ chain participates in the IL-7 receptor complex, we investigated whether IL-7 binds directly to the γ chain by the combination method of cross-linking and immunoprecipitation. The IxN/2b cells were incubated with ^{125}I-labeled IL-7 and cross-linked with disuccinimidyl suberate (DSS). Cell lysates were precipitated with TUGm3, which does not inhibit ligand binding, and analyzed by electrophoresis on an SDS-polyacrylamide gel. The precipitate included a radiolabeled molecule of 90 to 110 kD, presumably consisting of the γ chain and ^{125}I–IL-7 (Fig. 2).

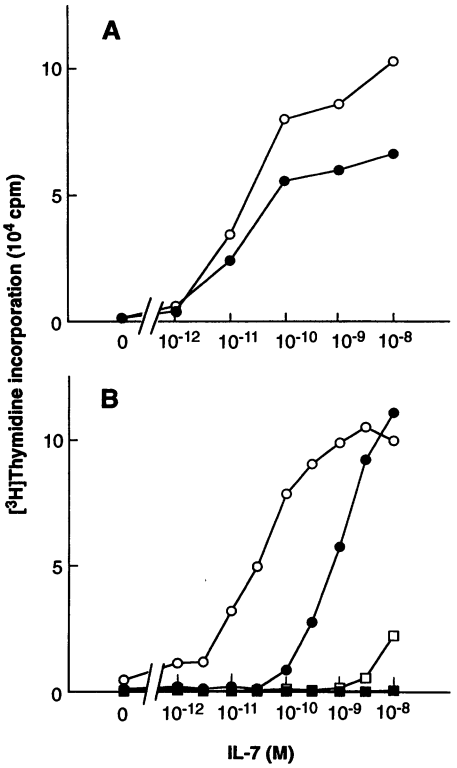

Fig. 1. Inhibition of IL-7–induced [^{3}H]thymidine incorporation by an antibody to the γ chain. (**A**) Spleen cells of C57BL/6 female mice (5W; Japan SLC Inc., Hamamatsu, Japan) were cultured in complete medium (RPMI 1640 with 10% fetal calf serum, 50 μM 2-mercaptoethanol, and antibiotics) with Con A (5 μg/ml) for 72 hours at 37°C, 7% CO_2 in humidified air. After washing, spleen cells (1×10^6) were further cultured for 72 hours with recombinant IL-7 (*12*). [^{3}H]Thymidine (1 μCi) was added 6 hours before harvesting on glass filters. Radioactivity was determined with a liquid scintillation counter. (**B**) An IL-7–dependent mouse pre–B cell line, IxN/2b (1×10^4), was similarly cultured for 48 hours in complete medium without Con A. Cells were labeled with [^{3}H]thymidine for 4 hours. Ascites mAbs at a 1:40 dilution were used: REY-7 (antibody to HTLV-I envelope protein gp46) (○), TUGm2 (antibody to mouse IL-2 receptor γ chain) (●), A7R34 (antibody to mouse IL-7 receptor) (□), and TUGm2 + A7R34 (■).

This molecule was not detected when TUGm2 or excess unlabeled IL-7 was added to the cells before cross-linking. This observation is similar to those shown previously with IL-2 and IL-4 binding to the γ chain (*7*) and indicates that interaction between the γ chain and IL-7 is possible, which suggests functional participation of the γ chain in the IL-7 receptor complex.

To gain insight into the effect of the γ chain on IL-7 binding, we did Scatchard analysis (*3*). The binding of IL-7 was measured with IxN/2b cells in the presence of the mAbs. With no specific mAbs, or in the presence of the control mAb REY-7, IxN/2b cells had two-phase binding plots: a

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high affinity of 79 pM, with 1505 binding sites, and a low affinity of 16 nM, with 35,160 binding sites (Fig. 3). Addition of TUGm2 reduced the affinity of the high-affinity site from 79 to 255 pM without strongly affecting the number of sites, whereas no change in the low affinity was apparent. In contrast, the cells treated with mAb A7R34, which is specific for the conventional IL-7 receptor, displayed only the low-affinity receptor. Results from treatment of cells with A7R34 together with TUGm2 did not differ from those from treatment with A7R34 alone. These results indicate that the γ chain is necessary for formation of the high-affinity IL-7 receptor complexes.

Two-phase plots were seen in IxN/2b cells even after the function of the γ chain was blocked by TUGm2. The moderately high affinity was close to the pseudo-high affinity associated with the functionally inactive IL-2 receptor complex consisting of the IL-2 receptor α and β chains (*4*). Thus the moderately high-affinity IL-7 binding site may be a complex of at least two subunits: the conventional IL-7 receptor and an unidentified molecule.

The low-affinity binding sites in the presence of A7R34 may be attributable to the presence of this unidentified molecule, which may be the component of the moderately high-affinity IL-7 receptor complex. The molecule may also account for intrinsic IL-7 binding sites detected on COS-7 (*14*) and THP-1 (*15*) cells, which are negative for the γ chain (*4, 16*). Exogenous expression of the conventional IL-7 receptor on COS-7 cells converted the low-affinity binding sites to the high-affinity binding sites (*14*)—presumably the moderately high-affinity binding sites. The γ chain alone did not show any appreciable IL-7 binding (*17*).

Our finding that the γ chain of the IL-2 receptor is a functionally essential subunit for the IL-7 receptor may be a step toward better understanding of the mechanisms that lead to development of normal lymphocytes and that lead to XSCID. Because lack of either IL-2 or IL-4 does not induce the XSCID phenotype, it seems difficult to account for XSCID by impairment of the function of the γ chain, which is used for the receptors for IL-2 and IL-4. In the context of involvement of γ chain mutations in XSCID, the conventional IL-7 receptor is expressed on $CD4^-CD8^-$ T lineage cells in the thymus (*12*), which also have the γ chain on their surface (*18*). Collectively, it may be that XSCID results from insufficient delivery of the IL-7 receptor–mediated signal as a result of the γ chain mutation in very early T precursors. Nevertheless, mice given antibodies against IL-7 and the conventional IL-7 receptor display, apart from profoundly reduced populations of T cells, complete depletion of B cells in the periphery (*12, 19*), which is distinct from the phenotype of human XSCID. Participation of IL-7 in lymphocyte development may be different in mice and humans, or another cytokine or cytokines requiring the γ chain as a receptor component may cause XSCID. The conclusion that the γ chain is involved in the IL-7 receptor complexes has been independently shown (*20*).

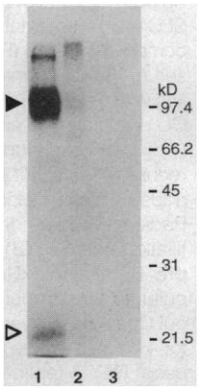

Fig. 2. Association of ^{125}I–IL-7 with the γ chain. Recombinant IL-7 was labeled with ^{125}I with the use of Enzymobead (Bio-Rad). After 2 hours of IL-7 depletion, IxN/2b cells (2×10^8 per lane) were surface-labeled with 1 nM ^{125}I–IL-7 and then chemically cross-linked with DSS (Pierce, Cambridge, United Kingdom). Cell lysates were prepared by solubilization with lysis buffer [25 mM tris-HCl (pH 7.5), 140 mM NaCl, 1 mM EDTA, 2 mM phenylmethylsulfonyl fluoride, 0.1% aprotinin, and 0.5% Nonidet P-40] and incubated for 4 hours at 4°C with complexes of TUGm3 bound to rabbit antibody to rat IgG (Zymed, San Francisco, California) coupled to AffiGel-10 (Bio-Rad). The immunoprecipitates were separated by electrophoresis on a 10% polyacrylamide gel (lane 1). In control experiments, diluted (1:10) TUGm2 ascites (lane 2) or 100 nM unlabeled IL-7 (lane 3) were added to the IxN/2b culture before addition of ^{125}I–IL-7. Closed and open triangles indicate 90- to 110-kD (γ chain cross-linked with ^{125}I–IL-7) and 25-kD (free ^{125}I–IL-7) molecules, respectively. The 90- to 110-kD bands may include a complex of ^{125}I–IL-7 and the conventional IL-7 receptor, in addition to the γ chain with ^{125}I–IL-7.

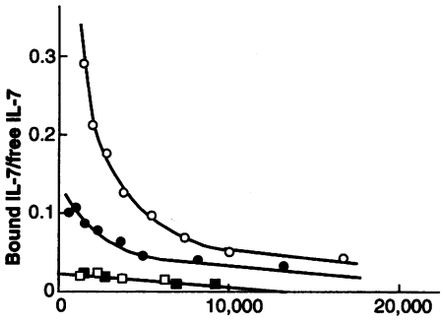

Fig. 3. Scatchard plot analysis of ^{125}I–IL-7 binding to IxN/2b cells. The plot was derived as described (*3*). The analysis revealed that IxN/2b expresses the high-affinity IL-7 receptors with a calculated dissociation constant of 79 pM and the low-affinity receptors with an apparent dissociation constant of 16 nM (IxN/2b cells alone, ○). With TUGm2 (100 μg/ml) (●), cells showed the moderately high-affinity receptor with an apparent dissociation constant of 255 pM and the low-affinity receptor with an apparent dissociation constant of 11 nM. Only the low-affinity receptor with a dissociation constant of 13 nM was seen in the presence of either A7R34 (50 μg/ml) alone (□) or both A7R34 and TUGm2 (■). Nonspecific binding was determined in the presence of more than 100 times excess unlabeled IL-7.

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